

1 **Beating** of hemp bast fibres: an examination of a  
2 **hydro-mechanical treatment on** chemical,  
3 **structural, and nanomechanical property evolutions**

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22

23 **Abstract**

24 In this study, a gradually increased hydro-mechanical treatments duration were applied to native  
25 hemp bast fibres with a traditional pulp and paper beating device (laboratory Valley beater). There  
26 is often a trade-off between the treatment applied to the fibres and the effect on their integrity. The  
27 multimodal analysis provided an understanding of the beating impact on the fibres at multiple  
28 scales and the experimental design made it possible to distinguish the effects of hydro- and hydro-  
29 mechanical treatment. Porosity analyses showed that beating treatment doubled the macroporosity  
30 and possibly reduced nanoporosity between the cellulose microfibrils. The beating irregularly  
31 extracted the amorphous components known to be preferentially located in the middle lamellae  
32 and the primary cell walls rather than in the secondary walls, the overall increasing the  
33 crystallinity of cellulose from 49.3 % to 59.1 %, but a non-significant change in the indentation

34 moduli of the cell wall was observed. In addition, **beating** treatments with two distinct mechanical  
35 severities showed a disorganization of the cellulose conformation, which significant dropped the  
36 indentation moduli by 11.2 GPa and 8.4 GPa for 10 and 20 minutes of Valley beater hydro-  
37 mechanical treatment, respectively, compared to hydro-treated hemp fibres (16.6 GPa). Pearson's  
38 correlation coefficients between physicochemical features and the final indentation moduli were  
39 calculated. Strong positive correlations were highlighted between the cellulose crystallinity and  
40 rhamnose, galactose and mannose as non-cellulosic polysaccharide components of the cell wall.  
41

## 42 **Keywords**

43 *Pile Valley **beater**, Plant cell wall, Nanoindentation, Porosity, Biochemistry,*  
44 *Natural fibre processing*  
45

## 46 **Abbreviations**

47 DVS:	dynamic vapour sorption;
48 HPAEC-PAD:	high-performance anion-exchange chromatography with
49	pulsed amperometric detection;
50 HPLC:	high-performance liquid chromatography;
51 MFA:	microfibrillar angle;
52 MIP:	mercury intrusion porosimetry;
53 <b>NCP's:</b>	<b>non cellulosic polysaccharides</b>
54 SEM:	scanning electron microscopy;
55 XRD:	X-ray diffraction
56	

## 57 **INTRODUCTION**

58 Lignocellulosic fibres have been of increasing interest in recent years in the field of  
59 agromaterials (Gallos et al. 2017). Their good mechanical properties make them a  
60 potential candidate to replace conventional man-made reinforcements in composite  
61 materials (Bourmaud et al. 2018; Mohanty et al. 2018; Wambua et al. 2003). Due  
62 to their multi-scale hierarchical structure, lignocellulosic fibres are akin to  
63 composite materials (Bourmaud et al. 2018). In plants, fibres are organized in  
64 bundles comprising several elementary fibres. Elementary fibres consist of a cell  
65 wall made mostly of cellulose ( $\beta$ -linked glucose unit chains) in a semi-crystalline

66 state (Turner and Kumar 2018). Cellulose microfibrils are parallel to each other,  
67 forming a microfibrillar angle (MFA) with the cell axis, and a fraction of them are  
68 connected to non-cellulosic matrix, usually called amorphous, made of  
69 hemicelluloses (mainly xyloglucan), pectins such as homogalacturonan (Morvan et  
70 al. 2003) and a small amount of lignins.

71 The cell wall of elementary lignocellulosic fibres is organized in concentric layers:  
72 from the outermost to the innermost, we find the primary cell wall; the secondary  
73 cell wall is composed of a xylan-rich layer type which is located on the outer  
74 periphery of a gelatinous layer type (also called S2); and finally a central lumen.  
75 The elementary fibres are linked together thanks to the pectic-rich middle lamella.  
76 The gelatinous layer type has a high cellulose content (80-90 %), high crystallinity  
77 index, and low angle of cellulose microfibrils, and in the case of hemp primary  
78 fibres, it is the thickest layer of the cell wall, more than 10  $\mu\text{m}$  (Chernova et al.  
79 2018).

80 Hemp bast fibres are used as composites in different industries, notably because  
81 hemp's cell wall is thick, with a reduced lumen (Beaugrand et al. 2017; Placet et al.  
82 2014), which gives it a high coarseness and is assumed to be non-collapsible  
83 (Westenbroek 2000). Hemp bast fibres are generally composed of 53-91 %  
84 cellulose, 4-18 % hemicelluloses, 1-17 % pectins, 1-21 % lignins in dry matter (Liu  
85 et al. 2017) and a low amount of wax present in the middle lamella (Thygesen et al.  
86 2006a). The large disparity in reported chemical composition can be explained by  
87 the fact that the analyses were not carried out at the same stage of fibre maturity or  
88 with the same measurement protocol (Bourmaud et al. 2018).

89 The mechanical properties of the cell walls are governed by several factors, such as  
90 the microfibrillar angle (Burgert and Keplinger 2013), the chemical composition  
91 (Lefeuvre et al. 2014), the crystallinity, the defect density (Andersons et al. 2009;  
92 Beaugrand and Guessasma 2015; Gourier et al. 2017; Hughes 2012; **Guessasma and**  
93 **Beaugrand 2019**) and the mesoporosity (pore size between 2 and 50 nm), even if  
94 the last factor is not yet well established at the elementary fibre scale. Arnould et  
95 al. (2017) mapped the stiffness of the cell wall of green flax stems by atomic force  
96 microscopy revealing a gradient in mechanical properties along the cross section at  
97 nanoscale. However, this gradient in properties is not observed in mature plants,  
98 except in cases of environmental accident, such as drought or lodging events.

99 The mechanical properties of short lignocellulosic fibre–reinforced thermoplastic  
100 composite materials are inherent to the properties of the fibres that make them up  
101 (Bourmaud et al. 2018; Shah 2013; Shah et al. 2016), including such variables as  
102 their reinforcement rate (Ausias et al. 2013), their orientation in the matrix (Tanguy  
103 et al. 2018), their defects (Beaugrand et al. 2017; Rask et al. 2012), their shape  
104 (Beaugrand and Berzin 2012; Berzin et al. 2014; Legland and Beaugrand 2013) and  
105 the fibre-polymer matrix adhesion (Le Moigne et al. 2018). However,  
106 lignocellulosic fibres are hydrophilic, i.e., they easily adsorb water molecules. This  
107 property, incompatible with the hydrophobic character of some thermoplastic  
108 matrices, such as polyolefins, can be a barrier to good fibre-matrix adhesion in  
109 composite materials. Many researchers have conducted work to improve fibre-  
110 matrix adhesion by applying thermal, mechanical and/or chemical pre-treatments  
111 to plant fibres. Numerous efforts to improve the properties of composites have been  
112 reviewed. Pickering et al. (2016) provide a synthesis of the various factors affecting  
113 the mechanical properties of natural fibre composites. More recently, Liu et al.  
114 (2017) reported the effects of many targeted pre-treatment techniques on hemp bast  
115 fibres intended for composite application.

116 Among the existing processes, the **beating** process is traditionally used in the pulp  
117 and paper industry. The effects of this treatment on wood are well known and  
118 described by Gharehkhani (2015), whereas much less knowledge is available on  
119 lignocellulosic plant fibres. The main ones are internal and external fibrillation -  
120 and consequently the opening of pore structure, increasing their swelling capacity -  
121 and the breakage and the straightening of the macrofibrils. However, also their  
122 chemical composition is changed by degumming cellulosic fibres, i.e., **beating**  
123 cellulose by removing the non-cellulosic fraction. To control the quality of the pulp,  
124 pulp and paper standard methods of characterization are used, such as the Schopper-  
125 Riegler degree (Dienes et al. 2005) or the water retention value (Cheng et al. 2010).  
126 Moreover, nanocelluloses are increasingly attracting materials science researchers  
127 and are being incorporated into formulations, and mechanical characterization  
128 techniques are applied to assess the properties of the fibres.

129 We tried to increase the external surface area of the **hemp fibre elements** by  
130 individualizing the bundles and producing microfibrils. To do this, we developed a  
131 soft hydro-mechanical treatment using a Valley beater. The effects of the treatment  
132 were revealed by analyses of the structural, chemical and nanomechanical features

133 obtained by SEM observations, polysaccharide analysis, porosity, cellulose XRD  
134 crystallography and nanoindentation. Process impacts were depicted on the fibres'  
135 structure and therefore on their mechanical properties. Finally, a correlation study  
136 identified the parameters that had the greatest impact on the nanomechanical  
137 properties of the fibres.  
138

## 139 **MATERIALS AND METHODS**

### 140 *Plant materials*

141 The native fibres used for this study were whole-plant hackled non-retted hemp  
142 fibres (*Cannabis sativa L.* variety Fedora 17). Hemp was cultivated and harvested  
143 in Champagne-Ardenne (France, 2012) and cut to an average length of 15  
144 millimetres by Fibres Recherche Développement (FRD, Troyes, France). Samples  
145 were stored in a dark environment at ambient temperature and humidity.

### 146 *Beating and drying of hemp fibres*

147 Before **beating**, hemp fibres were water-**soaked** for 10 min in a laboratory pulper at  
148 ambient temperature **to clean them and remove any impurities**. For this, 400 g of  
149 hemp fibre was immersed in 4 l of water (10 % w/v consistency) and stirred at a  
150 low rotational speed (510 rpm). Then, the cloudy water was removed and the bulk  
151 fibres put into the Valley beater (**described in TAPPI 200**) container. The container  
152 was then topped off with water to obtain a 2 % w/v consistency. The device was  
153 turned on for 5 minutes without counterweight to homogenize the suspension. The  
154 beating process started right after a weight of 4 kg was fixed to the lever **arm which**  
155 **corresponds to applying a force of 76.3 N upwards against the roll. The rotational**  
156 **speed of the roll was 510 rpm and the cutted edge length (CEL) calculated from**  
157 **Eq.1 was 34.7 m.r<sup>-1</sup>. From these two parameters and Number of crossing points**  
158 **(N<sub>cp</sub>) (Eq.2) between the roll and bedplate bars, we determined the cumulative**  
159 **number of crossing points (CN<sub>cp</sub>) per beating time (Table 1). All experiments were**  
160 **conducted with the same fibre concentration, counterweight and soaking time, only**  
161 **the beating time changes.**

$$162 \quad CEL = \frac{n_r \cdot n_s \cdot l_0}{\cos \alpha_R \cdot \cos \alpha_S} \quad (1)$$

$$163 \quad N_{CP} = \frac{CEL}{\pi \cdot D_R} \cdot \sin (\alpha_R + \alpha_S) \quad (2)$$

164 with  $n$  the number of bars,  $\alpha$  the angle of the bars, the R and S indices correspond  
 165 to the rotor (roll) and stator (bedplate) respectively.  $l_0$  and  $D_R$  are the width and  
 166 diameter of the roll respectively (Roux et al. 2009). The experimental was design  
 167 (Table 1) to make it possible to distinguish the effects of water (soaking step) and  
 168 hydro-mechanical treatment (beating).

169

170 The unbeaten native hemp fibres were the “ubH” sample, the beaten 0 minute hemp  
 171 fibres (only hydro-treated as hydro treatment) were the “bH\_0” sample, the hydro-  
 172 beaten 10 minutes hemp fibres were the “bH\_10” sample, and finally, the hydro-  
 173 beaten 20 minutes hemp fibres were the “bH\_20” sample (as the hydro-mechanical  
 174 treatment).

175

176 Table 1: Experimental design

Type	Code	Soaking time (min)	Beating time (min)	CN <sub>cp</sub>	SR°
Fibre consistency (w/v)	-	10%	2%	-	
Un-beaten hemp fibres (raw material)	ubH	0	0	0	n.a.
beaten 0 min Hemp fibres	bH_0	10	0	0	7 ± 0.3
beaten 10 min Hemp fibres	bH_10	10	10	1.6 10 <sup>6</sup>	8 ± 0.4
beaten 20 min Hemp fibres	bH_20	10	20	3.2 10 <sup>6</sup>	12 ± 0.4

177

178

179 The beaten fibre suspension was filtered on a homemade system made of a nylon  
 180 net having a pore size of 200  $\mu\text{m}$  that was attached to a wooden stretcher frame of  
 181 690×420 mm, i.e., a total surface area of 0.29 m<sup>2</sup> per frame. The frame was placed  
 182 in a large stainless-steel industrial sink. The suspension was spilled on it, and the  
 183 fibres were manually dispersed on a nylon net surface to keep them as separate as

184 possible. After that, the water was evacuated, and the nylon net surface was put  
185 inside a ventilated oven at 30 °C for 2 hours.

#### 186 *Schopper-Riegler degree (SR°)*

187 This rate is an indicator of the degree of **beating** of a fibrous suspension based on  
188 its ability to drain water that results directly from its surface conditions (fibrillation)  
189 and the swelling of the fibres. Thus, the higher the Schopper-Riegler degree, the  
190 more the pulp retains water and is more **beaten**. Unlike classical pulp, **in this study**  
191 **hemp fibres were beaten with a shorter duration than typical paper making time, in**  
192 **order to maintain** maximum integrity and mechanical properties. Therefore, to have  
193 a denser fibre mat, a suspension of 4 g of dry fibres (rather than 2 g) was diluted in  
194 1 l of water and introduced into the fill chamber of freeness tester. The water content  
195 of the suspension passed through a wire screen that retained the fibres and formed  
196 a mat. In the funnel, if the flow speed was too fast, the drained water exited by the  
197 lateral orifice and discharged in the corresponding graduated measuring cylinder.  
198 After a drying step, the absolute fibre mat mass was weighed to correct the SR° to  
199 2 grams per litre absolute dry weight. The SR° value of each treated fibre lot was  
200 averaged across 4 tests **(Table 1)**.

#### 201 *Scanning electron microscopy*

202 The surface appearance of the unbeaten and beaten fibres bundles was observed  
203 with a table-top scanning electron microscope (Hitachi TM-1000). This equipment  
204 did not require coating the fibres, and SEM images were taken at a magnification  
205 of 200 times and at an accelerating voltage of 15 kV.

#### 206 *Chemical composition*

207 The glycosidic linkages of polysaccharides were hydrolysed by two-step sulphuric  
208 acid attack to release monosaccharides and uronic acids. To open the structures, 10  
209 milligrams of milled fibres (sieved of 200 µm) was mixed with 125 **µl** of H<sub>2</sub>SO<sub>4</sub> 12  
210 M under stirring at room temperature for 2 hours, corresponding to the swelling  
211 step. Then, the H<sub>2</sub>SO<sub>4</sub> concentration was lowered by dilution at 1 M, and the  
212 mixture was heated at 100 °C for 2 hours to achieve the total hydrolysis.

213 Monosaccharides were separated and quantified by high-performance anion-  
214 exchange chromatography equipped with CarboPac Pa-1 Column. The detailed  
215 procedure was previously described by Herbaut (2018).

216 Cellulosic material is considered the sum of glucose (Glc), whereas the non-  
217 cellulosic polysaccharides (NCPs) content is the sum of the monomer units:  
218 arabinose (Ara), rhamnose (Rha), galactose (Gal), xylose (Xyl), mannose (Man),  
219 galacturonic acid (Gal Ac) and glucuronic acid (Glc Ac).

220 The acid-insoluble lignin content was quantified as described previously (Sharma  
221 et al. 2018). Milled samples (200 mg) were submitted to acid hydrolysis by mixing  
222 with 2 ml of a 12 M H<sub>2</sub>SO<sub>4</sub> solution for 2 h at room temperature. The acid solution  
223 was then diluted to a concentration of 2 M by addition of deionized water before an  
224 incubation of 3 h at 100 °C. The solid fraction was recovered, thoroughly washed  
225 and oven-dried at 105 °C to a constant weight. The Klason lignin content was  
226 calculated after correction for ash content, which was determined by calcination at  
227 550 °C for 4 h.

228 The protein content was estimated from the nitrogen content of the fibres.

229 Experiments were carried out using a EURO EA elemental analyser (Eurovector,  
230 Milan, Italy) equipped with a thermal conductivity detector. A precise mass of  
231 approx. 5 to 7 mg of dried-milled samples was weighed in a tin capsule, then  
232 hermetically sealed. Based on the Dumas method (Dumas 1831), the samples were  
233 fully flash-combustion oxidized, and nitrogen was converted into gaseous  
234 dinitrogen (N<sub>2</sub>). A correction factor (x6.25) was applied to nitrogen content to  
235 estimate the protein content (González López et al. 2010).

236 *Hygroscopic properties* by dynamic vapour sorption

237 The isotherms of the water vapour desorption/adsorption were determined using a  
238 dynamic vapour sorption (DVS) apparatus from Hiden Isochema Ltd. (UK). Nearly  
239 5 mg hemp fibres was placed into the microbalance's stainless-steel basket  
240 (precision of 0.1 µg), then placed hermetically in a double-jacket reactor connected  
241 to a thermoregulated water bath and equipped with temperature and relative  
242 humidity (RH) sensors to monitor them. Relative humidity was obtained with a flow  
243 mixture of wet and dry nitrogen. Once the sample was loaded into the DVS  
244 apparatus, the sorption/desorption sequence was started (10 % to 90 %RH then back  
245 to 10 %RH with a 10 % step) at a constant and regulated temperature of 20°C. Then,

246 a drying sequence was programmed to obtain the dry mass of the sample (4 h at 40  
247 °C then 8 h at 20 °C, under a flow of dry nitrogen). More details on the procedure  
248 are given by Guicheret-Retel (Guicheret-Retel et al. 2015). The moisture uptake  
249 was calculated as follows (Eq. 3) using the mass measured at the equilibrium  
250 moisture content for chosen RH ( $m_{moist}$ ) and the mass of dried sample ( $m_{dry}$ )

$$251 \quad \omega (\%) = \frac{m_{moist} - m_{dry}}{m_{dry}} \times 100 \quad (3)$$

252

### 253 *True Density*

254 True density measurements were carried out using the DVS apparatus from Hiden  
255 Isochema Ltd. (UK). Argon was used because it is an inert and heavy gas.  
256 Approximately 60 mg samples were used. The principle of this technique is to  
257 measure the apparent mass of the sample as a function of the pressure of argon  
258 applied from 1 to 10 bars with an interval of 1 bar. We obtained a linear curve of  
259 10 aligned points from which the slope was extracted and used to calculate the  
260 density of the sample using the buoyancy equation.

261 Before any measurement, a purge and dry cycle of the sorption microbalance was  
262 performed to obtain 100 % argon in the DVS device and to have a dried sample. To  
263 this end, we injected argon and put the system under vacuum approximately 20  
264 times, and then we maintained the sample under vacuum until its mass was constant.

### 265 *Fibre diameter distribution*

266 The fibre diameters were measured by a high-resolution 2D scanner (Epson V850  
267 pro) by scattering them on the scanner glass and taking care to keep them  
268 individualized. The images were acquired in 8-bit greyscale at a resolution of 4800  
269 dpi representing theoretically 5.29  $\mu\text{m}$  per pixel. The images were processed and  
270 analysed with ImageJ software. After a manual segmentation step by setting a  
271 threshold value, the fibres were labelled using the "binaryLabel8" plugin. The  
272 length of the fibres was obtained by computing their geodesic diameter, as  
273 implemented in the "MorphoLibJ" plugin (Legland et al. 2016; Legland and  
274 Beaugrand 2013). Their diameter was obtained from the largest circle that could be  
275 contained in the fibre. To plot the diameter distribution graphic, particles with a  
276 length strictly less than 200  $\mu\text{m}$  were excluded by numerical threshold. Also, by

277 convention, they are considered as ‘fine’ elements and not fibres (Ferreira et al.  
278 1999).

#### 279 *Thermogravimetric analysis*

280 Thermogravimetric analysis was performed on TGA Q800 (TA instruments Inc,  
281 USA) with a nitrogen flow ( $40 \text{ ml}\cdot\text{min}^{-1}$ ) from  $25 \text{ }^\circ\text{C}$  to  $1000 \text{ }^\circ\text{C}$  and a temperature  
282 ramp of  $10 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$ . The analysis was performed on approximately 20 mg of  
283 powder fibres ( $200 \text{ }\mu\text{m}$ ).

#### 284 *Mechanical properties of the cell wall*

285 The mechanical properties were assessed by nanoindentation. This technique  
286 requires the samples to be embedded in a resin. Before this step, the cell wall  
287 structure of hemp bundles was fixed using a paraformaldehyde (2 %)-  
288 glutaraldehyde (0.5 %) solution for 2 hours at room temperature. The fixed sample  
289 was dehydrated by successive ethanol solution baths (20 min) of increasing  
290 concentration until absolute grade. The samples were then soaked in successive  
291 solutions of ethanol/resin with an increasing ratio of resin until 100 % of resin.  
292 During those steps, the ethanol was gradually and then totally substituted by the LR  
293 White resin (Sigma-Aldrich). After that, the samples were placed inside a specific  
294 gelatine capsule and put in an oven. Finally, the resin polymerization was  
295 performed for 24 hours at  $60 \text{ }^\circ\text{C}$ .

296 The LR White capsule was cut in the transversal axis of the hemp bundles, and the  
297 excess resin around the fibre was manually removed. An ultra-microtome (Microm  
298 Microtech, HM 360) equipped with a diamond knife was used to smooth the sample  
299 surface and to obtain a roughness below  $0.5 \text{ }\mu\text{m}$ .

300 A Nanoindenter Xp equipped with a pyramidal indenter (Berkovich tip) was used  
301 to perform indentation measurements. The Nanoscope Analysis software enabled  
302 us to extract the indentation modulus and the indentation hardness from the load-  
303 unload curve of the specimen according to the method described by Oliver-Pharr  
304 method (Oliver and Pharr 1992). The indentation method parameters were fixed as  
305 follows: the maximum depth was 120 nm; the loading rate was  $1 \text{ }\mu\text{N}\cdot\text{s}^{-1}$ ; the dwell  
306 time at the maximum load was 20 s and the unloading rate was  $10 \text{ }\mu\text{N}\cdot\text{s}^{-1}$ .

### 307 *X-ray crystallography*

308 An ultra-centrifugal mill (Retsch ZM 200) was used to grind the hemp fibres to  
309 approximately 200 microns in length. The samples were scanned from  $2\theta = 5$  to 40  
310  $^\circ$  with a step size of  $0.03^\circ$  at  $2 \text{ s}\cdot\text{step}^{-1}$  at 30 kV and 20 mA under ambient conditions  
311 on a Siemens D500 diffractometer using  $\text{CoK}\alpha$  radiation. The crystallinity index  
312 was calculated using Eq. 4 according to the Segal empirical method (Segal et al.  
313 1959):

$$314 \quad C = \frac{I_{tot} - I_{am}}{I_{tot}} \times 100 \quad (4)$$

315 where  $I_{tot}$  is the intensity at the primary peak for cellulose I (at  $2\theta = 22.5^\circ$ ) and  $I_{am}$   
316 is the intensity from the amorphous portion evaluated as the minimum intensity (at  
317  $2\theta = 18.5^\circ$ ) between the primary and the secondary peaks.

### 318 *Mercury intrusion porosimetry*

319 To determine the pore size distribution and the total pore volume, an Autopore IV  
320 9500 (Micrometrics) was used. Approximately 150 mg of dried fibres were  
321 loaded in a suitable penetrometer. This technique is based on the non-wetting  
322 property of liquid mercury having high a surface tension ( $\gamma = 480 \text{ dyne/cm}$  at  $20^\circ\text{C}$ )  
323 and a high contact angle ( $\theta_{\text{air/mercury}} = 140^\circ$ ) on many surfaces. The mercury was  
324 gradually injected into the porous network thanks to an increasing pressure from  
325  $0.003 \text{ MPa}$  ( $178 \mu\text{m}$ ) to  $227 \text{ MPa}$  ( $0.003 \mu\text{m}$ ). The pore access ( $r$ ) invaded, which  
326 was determined by the Washburn equation (Washburn 1921), is inversely  
327 proportional to the exerted pressure (Eq. 5).

$$328 \quad r = \frac{-2\gamma\cos\theta}{P} \quad (5)$$

### 329 *Statistical analysis*

330 For chemical analyses, nanomechanical properties and true density measurements,  
331 the equality of variance (Fisher test) was verified. Student's t-test was carried out  
332 to compare the samples by pair, with a significance level of probability set at  $p <$   
333  $0.05$ . Pearson's correlation coefficients were evaluated between indentation moduli  
334 and chemical analyses, with a statistically significant p-value of  $< 0.05$ , using  
335 SigmaPlot 12.0 software (Systat Software, Chicago, USA).

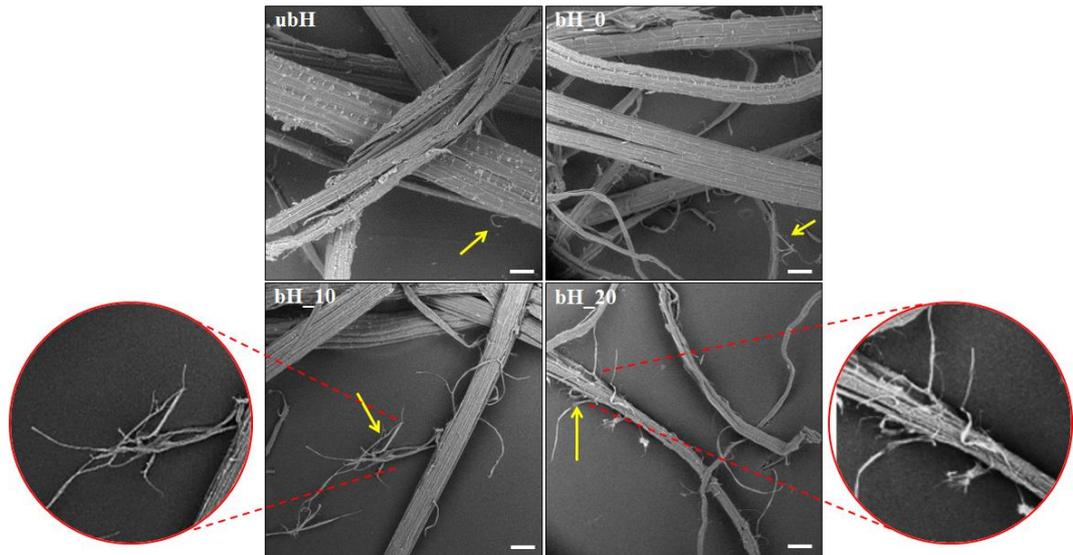
336

## 337 RESULTS AND DISCUSSION

### 338 *Structural changes*

339 The fibre suspensions were first characterized by the Schopper–Riegler test, which  
340 shows the progress of **beating** by the ability of the fibres to drain water. The **beaten**  
341 fibres had a SR° of 8 and 12 for bH\_10 and bH\_20 respectively (Table 1). This  
342 increase compared to **water-soaked** fibres (bH\_0) clearly showed that the fibre  
343 structure had been modified by mechanical **beating**, and the longer the beating, the  
344 greater the extent of modification.

345 Moreover, microscopic observations were conducted to visualize the fibre elements  
346 structural and topological natives state (ubH) and induced changes undergone by  
347 the hydro (bH\_0) and hydro-mechanical treatments (bH\_10 and bH\_20). The  
348 resulting images are shown in Figure 1. Untreated fibres (ubH) were thicker and  
349 had a rougher surface. After treatment, the surface of the fibres seemed to be  
350 cleaner, smoother but also finer and more defibrillated, especially with longer  
351 pretreatment times. The surface cleaning of fibre by an immersion step is long, and  
352 here our observations mostly confirm this. More interestingly, mechanical  
353 treatment (bH\_10 and bH\_20) resulted in defibrillation: the bundles dissociated into  
354 thinner fibres, and the upper layers of the fibres in the cell wall give rise to external  
355 fibrils or to fine particles if they dissociated from them. These phenomena are well  
356 known for wood (Gharehkhani et al. 2015; Page 1989) and for hemp (Wang et al.  
357 2007). In line with enhanced plant fibre composite mechanical properties,  
358 especially for short-fibre thermoplastic composites, the physical anchoring in the  
359 matrix mediated by fibrillation harboured by natural fibres is one promising route  
360 (Lee and Bismarck 2014; Zhong et al. 2011). One goal of this study was to generate  
361 such lateral fibrils along the fibres (see arrow in Figure 1), and the microscopic  
362 observations confirm the effectiveness of the Valley beater hydro-mechanical  
363 treatment.

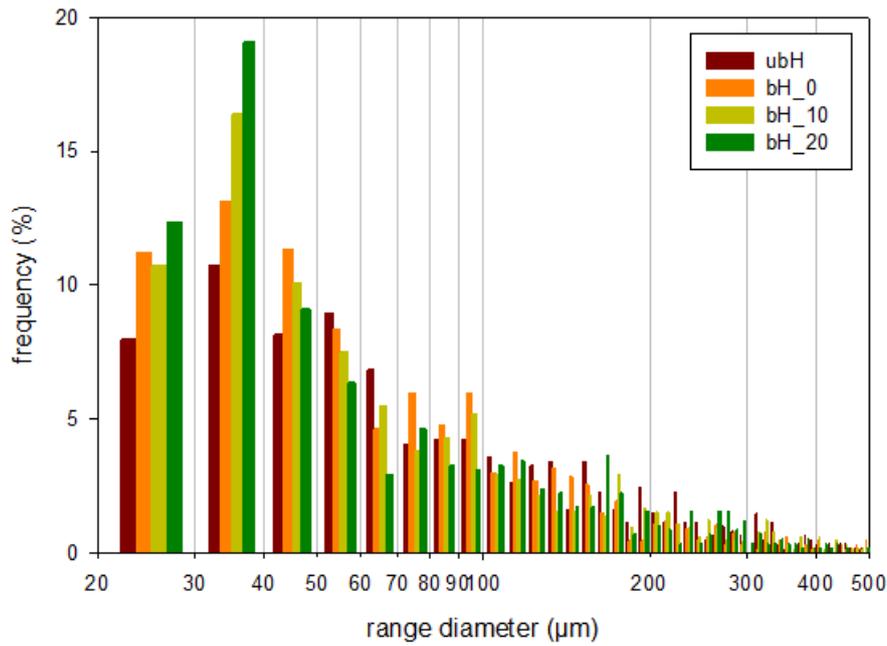


364

365 Figure 1: SEM micrographs. White scale bar equal to 50  $\mu\text{m}$ . Yellow arrows point the lateral  
 366 microfibrils more or less visible and amplified depending on the treatment severity.

367

368 Further details about the evolution of the morphologies were obtained by image  
 369 analysis. The average diameters of nearly 600 fibres with a minimum length of 200  
 370  $\mu\text{m}$  were measured for each sample to have a global representation of the fibre  
 371 population (Figure 2). The majority of the untreated fibres (ubH) extend to  
 372 diameters between 20 and 60  $\mu\text{m}$ . The same trend was observed for water-soaked  
 373 fibres (bH\_0), with a slight enrichment of this fraction, suggesting that the soaking  
 374 step has allowed the beginning of individualisation of some bundles. This would be  
 375 in agreement with the water-soluble non-cellulosic polymers extracted from the  
 376 fibre elements, in particular from the middle lamella that ‘glued’ the elementary  
 377 fibre together. This range was gradually reduced to 20-40  $\mu\text{m}$  for mechanically  
 378 treated fibres with a 50 % enrichment compared to native fibres (ubH). As the fibres  
 379 were beaten as the mechanical treatment progresses, these diameter results  
 380 corroborated the abovementioned microscopic observations. It should be noted that  
 381 the method of measuring the diameter by the largest inscribed circle that can be  
 382 contained in the fibre may slightly overestimate the diameter, especially for the  
 383 most fibrillated fibres. Indeed, we noticed that this larger inscribed circle tended to  
 384 be at the branching points.



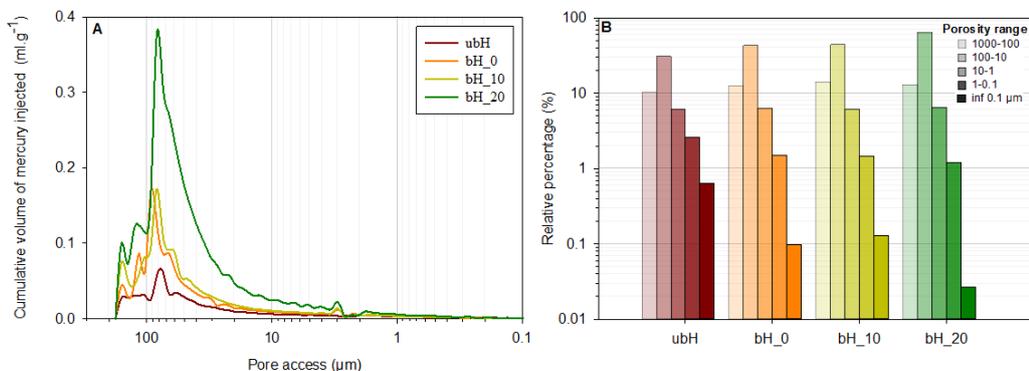
385

386 Figure 2: Diameter distributions of hemp fibres greater than 200 µm in length.

387

### 388 *Porosity and swelling*

389 With the objective to estimate the impact of soaking and beating on the porous  
 390 structure and surface pore area of hemp fibres, a porosity analysis was performed  
 391 by mercury intrusion porosimetry (MIP). The mercury porosity obtained on the  
 392 native fibres (ubH) was 49.8 %. In the water-soaked fibres (bH\_0), the porosity  
 393 was higher with a value of 62.6 %. The total porosity increased with the mechanical  
 394 treatment applied to the fibres (64.8 % for bH\_10 and 83.3 % for bH\_20).  
 395 Regarding the pore distribution (Figure 3A), the mean pore access for the native  
 396 fibres was approximately 75 µm that corresponded to the interfibre spaces.



397

398 Figure 3: Incremental volume of mercury injected as a function of pore access ( $\mu\text{m}$ ) (3A) and  
 399 range of porosity distribution in relative percentage for each fibre sample (3B). The legend of the  
 400 Figure 3B shows the intensity gradient corresponding to each porosity range.

401

402 This mean pore access was up to 90  $\mu\text{m}$  for the water-soaked fibres and up to 83  
 403 microns for the mechanically treated fibres. A small peak approximately 3  $\mu\text{m}$   
 404 which intensity increased with the treatment constituted the fibres microporosity  
 405 could correspond to the lumens. They were defined as hollow and elongated  
 406 cavities with transversal diameter between 0.5-10  $\mu\text{m}$  (Del Masto et al. 2017; Placet  
 407 et al. 2014). This observation obtained with the MIP also matches well with the  
 408 lumen description visualized by X-ray nanotomography (Beaugrand et al. 2017).  
 409 Considering the relative percentages of the pore access ranges (Table 2), we can  
 410 see that it was the 10-100  $\mu\text{m}$  range that increased significantly, i.e., it increased  
 411 by 30 % for bH\_0 and bH\_10 and almost doubled for bH\_20 compared to native  
 412 fibres.

413 Table 2: Mercury intrusion porosimetry results: total porosity and apparent skeletal density. True  
 414 density obtained by DVS. True densities not having the same letter were significantly different  
 415 ( $p < 0.05$ ).

416

	Total porosity (%)	Apparent skeletal density ( $\text{g}\cdot\text{ml}^{-1}$ ) 1)	True density
ubH	49.8	1.379	$1.535 \pm 0.004^a$
bH_0	62.6	1.280	$1.541 \pm 0.004^a$
bH_10	64.8	1.254	$1.534 \pm 0.005^a$
bH_20	83.3	1.453	$1.550 \pm 0.004^a$

417

418 This reflects the densification of porous networks, both intrafibre and interfibre,  
 419 through bundle individualization and the formation of lateral microfibrils that can  
 420 become entangled and sometimes in loops as can be observed in the SEM images  
 421 (Figure 1). Pores smaller than 0.1 microns almost completely disappeared as soon  
 422 as the fibres were soaked and dried. These nanopores corresponded to the pores  
 423 between the microfibrils (Meng and Ragauskas 2014), so the soaking/drying cycle  
 424 imposed on the fibres tightened the cellulose microfibrils between them.

425 In addition, water sorption tests using DVS were conducted to learn about the  
 426 qualitative evolution of mesoporosity. Indeed, in the case of natural fibres, a  
 427 hysteresis was observed during the desorption phase, reflecting the presence of  
 428 mesopores in the fibre structure (Céline et al. 2013; Hill Callum et al. 2009).  
 429 Recently, Chen et al. (2018) have provided, through simulations, a better  
 430 understanding of the role of hydrogen bonds involved in the hysteresis observed in  
 431 sorption-induced swelling of soft nanoporous polymers.

432 The isotherms were analysed by two means: (i) the loop isotherm area (Table 3)  
 433 resulting from subtraction of the desorption curve area from the sorption curve area  
 434 (Figure 4A), for which areas were estimated by the trapezoidal rule; and (ii) the  
 435 hysteresis curves, i.e., the difference in water uptake ( $\omega$ ) between desorption point  
 436 and sorption point as a function of relative humidity (Figure 4B).

437

438

439

440

441

442 Table 3: Characterization results of untreated and treated hemp fibres: (i) loop area of the  
 443 sorption/desorption curve, (ii) nanoindentation modulus and (iii) crystallinity. Values not having  
 444 the same letter were significantly different ( $p < 0.05$ ).

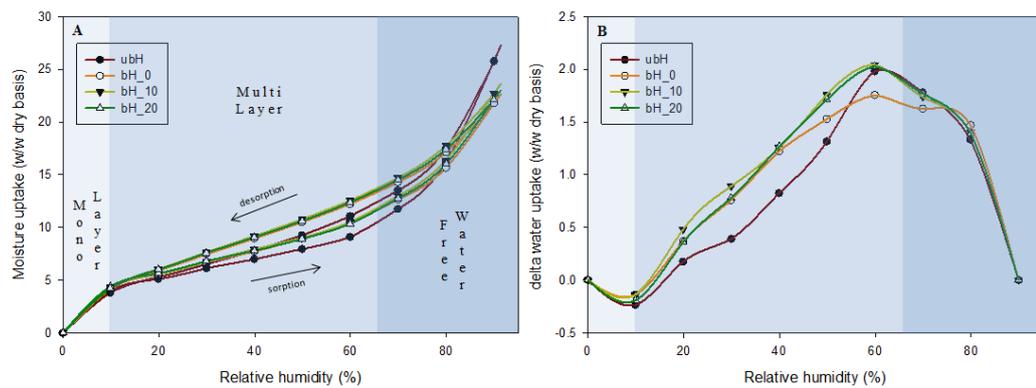
445

	Loop area ( $\omega\% \cdot \text{RH}\%$ )	Total sugar content (% dry matter)	Crystallinity (%)	Indentation modulus from unload (GPa)
ubH	75.2	$84.3 \pm 7.6^a$	49.3	$15.1 \pm 2.7^a$
bH_0	86.1	$86.5 \pm 4.2^a$	59.1	$16.6 \pm 2.5^a$
bH_10	94.4	$85.1 \pm 3.1^a$	56.6	$11.2 \pm 1.0^b$
bH_20	91.7	$91.5 \pm 2.3^b$	54.1	$8.4 \pm 1.0^c$

446

447 Water sorption and desorption isotherms are shown in Figure 4A. The curves of the  
 448 treated and untreated fibres followed the same pattern, and three curve parts could  
 449 be distinguished. At low relative humidity (RH), up to approximately 10 %, the  
 450 water molecules were expected to bind to the surface of the sample in a monolayer  
 451 by van der Waals forces. This involves the hydroxyl groups of hemicelluloses and  
 452 amorphous cellulose as well as the carboxylic functions of pectin (Céline et al.

453 2014), all present in hemp fibre (Placet et al. 2017). Notice that for all samples, the  
 454 curves where the moisture content was below 10 %RH could be superimposed.



455  
 456

457 Figure 4: Sorption-desorption curve: moisture uptake expressed in percentage of dry matter (4A).  
 458 Hysteresis curve: delta moisture content (4B) as a function of relative humidity. The delta is the  
 459 subtraction of the moisture uptake in the desorption phase from the moisture uptake in the sorption  
 460 phase, at equivalent relative humidity.

461

462 When the entire surface was saturated, the molecules continued to adsorb and pile  
 463 up on the monolayer, forming a multilayer, until all accessible voids were filled.  
 464 Above approx. 65-70 %RH, water molecules aggregated in a free liquid state. At  
 465 relative humidity between 10 and 65 %, treated fibres adsorbed a greater quantity  
 466 of water than untreated fibres, meaning that the porous structure of the fibres had  
 467 opened, probably due to the extraction of some cell wall oligo-polysaccharides by  
 468 water-assisted mechanical removal, as shown by MIP. This was confirmed by  
 469 chemical analysis; see the next section. Thus, physical water sorption predominated  
 470 rather than chemo-sorption in this range, because although water absorbing  
 471 molecules (hemicelluloses/pectins) were extracted during treatment, more sorption  
 472 took place, which could be explained by mesoporosity overcompensation.

473 Regarding the isotherm curves of Figure 4A, at 90 % RH, untreated fibres  
 474 absorbed 25.7 % water compared to the 21-22 % moisture content of treated fibres.  
 475 The loop areas (Table 3) confirmed that the three treated fibres had a more  
 476 noticeable hysteresis response than the unbeaten fibres, with an area of 86.1, 94.4  
 477 and 91.7  $\omega$  % · RH%, which were 15 %, 25 % and 22 % increases, for bH\_0, bH\_10  
 478 and bH\_20, respectively. Thus, accessibility to sorption sites begins at the soaking  
 479 stage and is accentuated with mechanical treatment. In other words, hemp fibres  
 480 had a swelling capacity increased by beating, which seems to follow a similar

481 behaviour to that reported for lignocellulosic pulp (Pönni et al. 2012). This can  
482 probably be connected to cell wall polymers water plastization and arguably by the  
483 multiplication of defects (kink bands, fibrils...), where one could expect more  
484 water-binding sites.

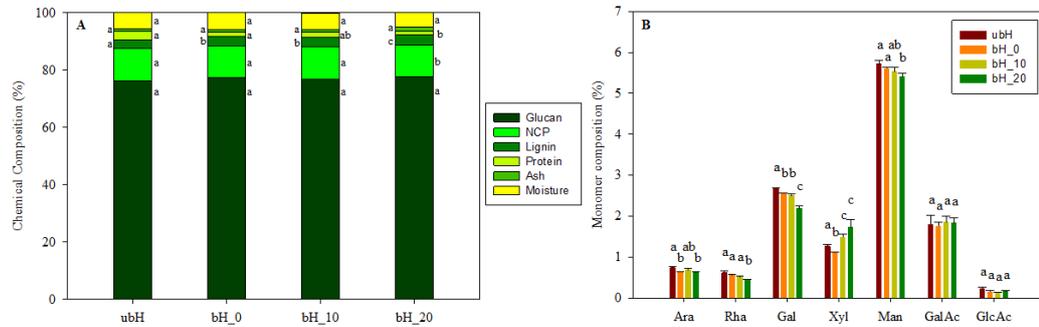
485 Even if isotherm curves had a similar trend (Figure 4A), the hysteresis curves  
486 (Figure 4B) yielded more detail concerning the pore opening/closing mechanism  
487 induced by mechanical treatment. At a relative humidity between 10 and 40 %, the  
488 three treated fibres were almost superimposed and had a much higher adsorption  
489 capacity than native fibres. Beyond that, the behaviour differed for bH\_0 fibres,  
490 since between 40 and 70 %, it adsorbed less water than bH\_10 and bH\_20 fibres  
491 and even less than native fibres at RH between 55 and 70 %. From these  
492 observations, we can deduce that water treatment opens smaller pores, unlike  
493 hydro-mechanical treatment, which contributes to the opening of larger sites that  
494 can adsorb water. Westenbroek (2000) mentioned that the small size of the lumen  
495 makes the hemp fibres uncollapsible. However, the fact that the delta water content  
496 of bH\_0 was lower than that of native fibres between 55 and 70 % RH shows that  
497 there was some closure of porous cavities.

498 Concerning the true density measured by DVS, all the sampled had a true density  
499 between 1.53 and 1.55 g.cm<sup>-3</sup>. The apparent skeletal density measured by MIP took  
500 into account the internal porosity of the fibre, explaining that the measured density  
501 was lower than true density, as recently explained by Legall et al. (2018) for flax  
502 fibres. Because the apparent skeletal density of the most treated fibres in the bH\_20  
503 group was raised to 1.453 while the others decreased (1.28 for bH\_0 and 1.25 for  
504 bH\_10) compared to untreated hemp fibres (1.38), no particular trend could be seen.

### 505 *Chemical composition*

506 To investigate chemical composition evolution due to treatment, an analysis of the  
507 major dry matter based component of the hemp fibre was performed. The  
508 polysaccharide, proteins and lignin contents were assessed (Figure 5A). The  
509 untreated hemp fibres used in our study (ubH) were composed mainly of  
510 polysaccharides, 86 % of the dry mass in total, including 75 % cellulose and 11 %  
511 non-cellulosic polysaccharides (NCPs), which respectively corresponded to 87 %  
512 and 13 % of the relative content of total polysaccharide. Additionally, the acid-  
513 insoluble lignin fraction (called Klason lignin) represented 2.9 % of the dry matter,

514 similar to the protein content (3 %). These results of chemical composition of hemp  
 515 fibres are in agreement with those of the literature when comparable analytical  
 516 techniques were used (Crônier et al. 2005; Placet et al. 2017). Indeed, it is  
 517 noteworthy that the biochemical technique used here may give contrasting results  
 518 for the same sample.



519

520 Figure 5: (A) Stacked bar charts of chemical composition expressed as the dry matter content. (B)  
 521 Monomer composition expressed as the percentage of total polysaccharide. Bars not having the  
 522 same letters are significantly different ( $p < 0.05$ ).

523

524 When the fibres were soaked, the total sugar content slightly and gradually  
 525 increased with the mechanical treatment time, and finally became significantly  
 526 different at 20 minutes of beating (see Table 3). The opposite trend was observed  
 527 for proteins: from the soaking stage and up to 10 minutes of **beating**, their content  
 528 was significantly reduced by half to reach 1.4 % in 20 minutes of **beating**. In fact,  
 529 the aqueous suspension of hemp fibres caused the solubilization of proteins and  
 530 other low-molecular-weight organic compounds, furthering a slight enrichment of  
 531 cellulosic and phenolic fractions comparing to **unbeaten** hemp fibres (ubH). Some  
 532 proteins are located in the middle lamellae and primary cell walls and are arguably  
 533 water soluble. In contrast, another protein compartment is non-water soluble  
 534 because it interacts strongly with the cell wall network and directly with cellulose  
 535 microfibrils. Those are structural proteins and are generally glycoproteins from the  
 536 arabino-galactan protein superfamily, such as fasciclin-like arabinogalactan  
 537 proteins (Guerriero et al. 2017). Moreover, the peeling of the fibre cell wall by the  
 538 beating process increased the exchange surface with water and thus seemed  
 539 amplified this solubilization by removing more soluble components, letting more  
 540 polysaccharide polymers in the bH\_20.

541 One would have expected the lignin to be extracted too, despite its low level in  
542 hemp fibres but greater enrichment in the middle lamella due to its location.  
543 However, its content evolved like the polysaccharide content. According to Akmar  
544 et al. (2000), lignin can neither be modified nor extracted by simple mechanical  
545 treatment such as the one we choose in this study.

546 We can see also that the relative proportion of the total non-cellulosic  
547 polysaccharide fraction was not affected under the three conditions (Figure 5A).  
548 However, looking in more detail at the monosaccharide composition of NCPs, the  
549 repartition of some monomer units was slightly contrasted, depending on the  
550 applied treatment. Arabinose, galactose and mannose units were significantly  
551 declined by the **soaking step**. Then, when comparing the bH\_0 with both beaten  
552 samples, we can see first that the chemical composition of bH\_10 was similar to  
553 bH\_0 except for xylose, which increased from 1.1 % to 1.5 % of total  
554 polysaccharide. Under the most drastic treatment, bH\_20, arabinose, rhamnose,  
555 galactose, and mannose were reduced, possibly causing an enrichment of xylose.  
556 The solubilization of these monomers, mainly inherent in the middle lamella and  
557 the primary **cell** wall, facilitated the delamination of fibre bundles into unit fibres,  
558 which is in line with the abovementioned diameter changes.

559 Additionally, special attention should be paid to the xylose content because in  
560 contrast to the other NCPs components, it is the only one with an enriched content.  
561 The quantified xylose in our study certainly could come from a xylan-rich layer  
562 (located in the secondary cell wall), and the latter would not be affected by soaking  
563 and **beating**. Interestingly, a xylose enrichment has also been reported during  
564 bamboo processing by bleaching and caramelization procedures (Sharma et al.  
565 2018); those authors hypothesized that the xylan backbone was covalently bonded  
566 with other cell wall polymers, such as lignin via hydroxycinnamic moieties (such  
567 as ferulic acids), to form a cross-linked network, which limited chemical access.

568 Galacturonic acid is often related to pectin, and in fruit it is often related to  
569 rhamnogalacturonan I. Curiously, with hemp bast fibre elements, the level of  
570 galacturonic acid was constant between all the conditions, suggesting that hemp  
571 bast fibre pectin may have limited susceptibility to peeling treatment (Placet et al.  
572 2017). Glucuronic and galacturonic acids were constant and not enriched, meaning  
573 that they seemed removed, but the significance of this finding is questionable. To  
574 close the polysaccharide examination, we have to recall that the processes used here

575 for our samples was unheated, non-alkaline and without barometric stress, leading  
576 us to assume that this explains the relatively low rates of hemicelluloses and pectins  
577 extracted from hemp fibres.

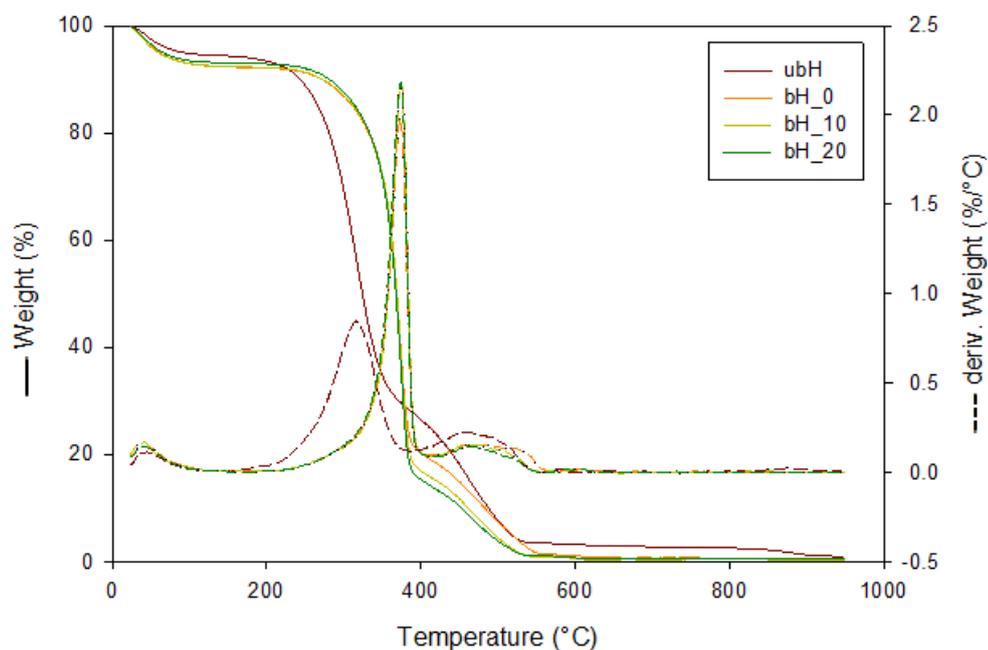
#### 578 *Crystallinity of cellulose*

579 According to equation (1), the crystallinity index of untreated fibres (ubH) was 49.3  
580 % (Table 3) which is in line with the expected values for hemp bast fibres (Dai and  
581 Fan 2010; Shah 2013). The bH\_0 sample had a crystallinity of 59.1 %, which  
582 represented an increase in the index of 10 compared to ubH. Yao and co-workers  
583 (Yao et al. 2018) have studied the hydro-thermal pretreatment effect on the structure  
584 of corn stover cellulose and highlighted changes in cellulose conformation by  
585 rearrangement of hydrogen bonds during decrystallisation-recrystallization phases  
586 of cellulose occurring during pretreatment. Thus, possible polymer rearrangements  
587 may have contributed to the increased crystallinity index, due to the water  
588 environment and the closure of nanopores between the cellulose microfibrils, as  
589 suggested by the porosity results above. A slight extraction of amorphous  
590 components could increase the crystallinity index (Xu et al. 2013), but probably to  
591 a lesser extent compared to the rearrangement of cellulose microfibrils.

592 The hydro-mechanical treatments resulted in a drop in the crystallinity index, index  
593 values of 56.6 % and 54.1 % were obtained for bH\_10 and bH\_20, respectively.  
594 Thus, mechanical treatment could have disrupted the crystalline structure of  
595 microfibrils by inducing dislocations. These dislocation zones were defined as areas  
596 of the fibre cell wall where the direction of the microfibrils (the angle of the  
597 microfibrils) differed from the angle of the microfibrils of the surrounding cell wall  
598 (Thygesen et al. 2006b). To support this hypothesis, Dai and Fan (2011) used FTIR  
599 spectroscopy to quantify the crystallinity index of two kinds of hemp samples, one  
600 without and the second with dislocations. They found that the crystallinity index  
601 was 48.4 % for the hemp without dislocations and 41.3 % for those within  
602 dislocation regions, showing a significant reduction in the crystallinity due to the  
603 dislocations.

#### 604 *Thermal stability evolution*

605 The evolution patterns of weight loss and their derivatives are shown in Figure 6.



606

607 Figure 6: Thermogravimetric analysis curve. (-) percentage of weight and (---) derivative of weight  
 608 as degradation temperature (°C).

609

610 The decomposition steps of lignocellulosic biomasses have been extensively  
 611 studied (Carrier et al. 2011; Stefanidis et al. 2014). Even if the interactions between  
 612 hemicelluloses, cellulose and lignin are complex and have an impact on the thermal  
 613 stability of the samples, a common pattern emerges. Indeed after the dehydration  
 614 stage (peak I), the least stable compounds, pectins and hemicelluloses decomposed  
 615 between 210 and 350 °C, followed by cellulose (peak II) up to 400 °C. Pectins and  
 616 hemicelluloses having semi-crystalline polymers are often considered an  
 617 amorphous polymer *in planta*. Lignin is a more complex compound which is rich  
 618 in aromatic units and degrades at a high temperature, between 400-500 °C (peak  
 619 III), but starts slightly from 100 °C onwards, according to its condensation level. At  
 620 the end of the pyrolysis, what remains is called the ashes. The temperatures of  
 621 thermal degradation obtained are shown in Table 4 and are in agreement with the  
 622 above temperature ranges and those described in previous studies (Mazian et al.  
 623 2018).

624 Regarding the moisture content related to peak I (Table 4), we can note that fibres  
 625 contained similar moisture content.

626 Table 4: Thermogravimetric analysis peaks. Weight loss and mass residue (W%) expressed in  
 627 percentage and temperature (T°) expressed in °C.

	Peak I		Peak II		Peak III		Residue
	W%	T°	W%	T°	W%	T°	W%
ubH	5.9	37.9	66.3	308.2	24.0	454.5	0.95
	± 0.35	± 5.0	± 0.47	± 0.62	± 0.37	± 0.2	± 0.07
bH_0	5.8	39.5	74.9	362.7	17.2	484.4	0.59
	± 0.59	± 2.7	± 1.19	± 0.3	± 0.23	± 7.90	± 0.06
bH_10	7	40.5	77.8	367.4	14.8 ±	477.7	0.78
	± 0.94	± 0.01	± 0.1	± 6.79	0.15	± 6.08	± 0.13
bH_20	6.0	38.5	79.9	361.8	13.0	457.6	0.6
	± 0.10	± 2.7	± 0.17	± 0.2	± 0.1	± 1.79	± 0.16

628

629 Additionally, the thermogravimetric analysis (Figure 6) shows that the cellulose  
 630 peak (peak II) was narrowed and shifted in the three treated fibres compared to the  
 631 untreated sample ubH. This could be explained by the extraction of water-soluble  
 632 components and thus higher cellulose crystallinity. The maximum temperature of  
 633 degradation of the cellulose (peak II) was 362.7 °C for the water-soaked fibres  
 634 against 308.2 °C for the raw fibres ubH, as shown in Figure 6, so it was necessary  
 635 to bring more thermal energy to degrade the cellulose. According to a study on  
 636 wood conducted by Giummarella et al. (2017), it would seem that the extraction of  
 637 hemicelluloses on the surface of cellulose microfibrils is in favour of stronger  
 638 interaction between lignin and cellulose. Even if hemp fibres have a lower lignin  
 639 content compared to wood, it is arguable that new interactions between  
 640 macromolecules of the cell wall involving cellulose were formed, resulting in a  
 641 higher thermostability, as shown experimentally in Figure 6, where we observed a  
 642 shift of temperature from 354.5°C to 484.4°C for hydro-treated fibres bH\_10  
 643 compared to ubH at the maximum temperature of degradation of peak III. Then, we  
 644 can see that for bH\_10 and bH\_20, this temperature gradually decrease (477.7 and  
 645 457.6 °C) and simultaneously their percentage of weight loss also (14.8 and 13%).  
 646 The mass of degraded cellulose gradually increased from 66.3 % to 79.9 %,  
 647 confirming that the cellulose compartment was modified. At the end of pyrolysis,  
 648 the ash (residue) was less than 1 % for each sample.

649

650 *Mechanical properties by nanoindentation and correlation studies*

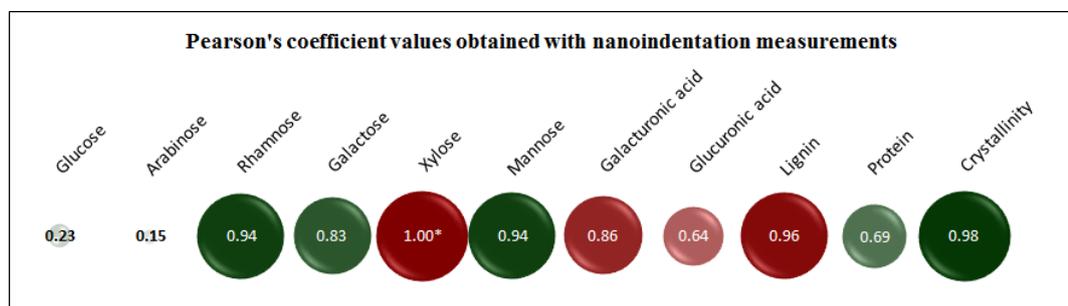
651 Nanoindentation tests were carried out to compare and discuss the impact of the  
652 treatments on the stiffness of the hemp fibre cell walls (Table 3). It is important to  
653 recall that with this technique, only the S2 sublayer, which is the most richly  
654 cellulosic layer, can be reasonably investigated due to the small thickness of the  
655 other sublayers. Due to the high mechanical anisotropy of hemp cell walls and  
656 specific loading mode, nanoindentation can not clearly give absolute mechanical  
657 data (Bourmaud and Baley 2009). Nevertheless, it is a suitable tool to compare  
658 mechanical properties of plant cell walls, being the only method to obtain  
659 mechanical information at the cell wall scale.

660 Untreated fibres had a  $15.1 \pm 2.7$  GPa indentation modulus, which, is in the same  
661 range than literature data; for, example Marrot et al. (2013) measured indentation  
662 modulus between 15.3 and 16.1 GPa in primary hemp fibres . However, this  
663 stiffness started to drop as soon as the mechanical treatment was applied, decreasing  
664 drastically to  $11.2 \pm 1.0$  and  $8.4 \pm 1.0$  GPa at 10 and 20 minutes of beating,  
665 respectively, which made sense with the loss of crystallinity of cellulose.  
666 Adusumalli (2014) also observed this decrease in cell wall nanoindentation stiffness  
667 after bleaching and beating treatment on wood pulp fibres. Due to the solicitation  
668 mode, the nanoindentation results are strongly impacted by the plant fibres MFA  
669 (Jäger et al. 2011), the indentation modulus being particularly sensitive to cellulose  
670 and the hardness to non-cellulosic polymer matrix. Indeed, the presence of  
671 porosities in these latter may greatly impact the hardness, due to the calculation  
672 method, which considers a theoretical contact area that may be false if the sample  
673 is damaged, as it may have been here.

674 The decrease in the indentation modulus can also be attributed to an increase in  
675 microfibrillar angle, as Tze et al. (2007) have shown for wood cell wall and as  
676 Burgert and Keplinger (2013) mentioned in their review about nanomechanical  
677 tests for plant cell walls. Once again, the loading mode is particularly sensitive to  
678 macrofibrils stiffness but also to changes into MFA. In our case the important  
679 increase into porosity content (Table 2) is in favour of changes into MFA values  
680 and possible evolution of cell wall stiffness, as evidenced by nanoindentation  
681 results.

682 Based on bibliographic data on the chemical composition (cellulose,  
683 hemicelluloses, lignin, pectin, waxes) and physical properties (diameter, MFA,

684 length, density, moisture gain) of 21 types of natural fibres, Komuraiah et al. (2014)  
 685 conducted a Pearson's correlation study to reveal the influence of these  
 686 physicochemical characteristics on their macro-mechanical properties (tensile  
 687 strength, specific strength, Young's modulus, specific Young's modulus, failure  
 688 strain). Here, we applied this correlation test in an attempt to establish which  
 689 chemical and structural modifications of the fibres undergoing mechanical  
 690 treatment had the greatest impact on their indentation modulus, and to give  
 691 additional data for the analyse and interpretation of mechanical tendencies noticed,  
 692 correlation studies were carried out using Pearson's correlation test (Figure 7).  
 693



694  
 695 Figure 7: Pearson's correlation coefficient between nanoindentation measurements and treated  
 696 fibre-related features. Disc diameter and colour shade display the strength of the correlation. Both  
 697 positive and negative correlations are presented using the absolute value of the coefficients.  
 698 Positive and negative correlations are displayed in green and red, respectively.  
 699

700 The mechanical properties, and especially the indentation modulus of the plant cell  
 701 wall are mainly conferred by the cellulose microfibrils. Cellulose being a chain of  
 702 glucose units, one would expect that the indentation modulus would be highly  
 703 correlated with glucose content but also by properties and structure of cellulose.  
 704 Here, the hydro-mechanical treatment applied to the fibres did not significantly  
 705 impact the glucose content but gradually disorganizes the cellulose chains, causing  
 706 both the crystallinity index and the indentation modulus to drop, these two  
 707 parameters being strongly positively correlated (Figure 7). Moreover, the increase  
 708 of porosity content within the cell wall is in favour of a drop of mechanical stiffness,  
 709 especially due to the solicitation mode which may induce buckling of cellulose  
 710 macrofibrils during indentation.

711 Interestingly, a strong correlation with the indentation modulus was obtained for  
 712 rhamnose (0.94), galactose (0.83) and mannose (0.94), whereas these are mainly  
 713 monomers composing pectins and hemicelluloses. In our study configuration, this

714 means that the indentation modulus decreased in proportion to the contents of these  
715 3 non-cellulosic monomers. Pectins and hemicelluloses form a very complex  
716 network with microfibrils and act as a glue in this structure. Different authors have  
717 underlined the important function of non-cellulosic components into plant cell wall  
718 stiffness, favouring the link and cohesion between cellulose microfibrils  
719 (Bourmaud and Baley 2009; Lefeuvre et al. 2015). Thus, the possible solubilization  
720 of such compounds may cause a relaxation of the microfibrils' structure, possibly  
721 resulting in an MFA rearrangement as well as a loss of cell walls mechanical  
722 properties.

723 On the other hand, xylose (1\*) and lignin (0.96) had strong negative correlations  
724 with nanomechanical properties. In our case this shows a recalcitrance to extraction  
725 by water and the soft mechanical beating. Thus, the positive correlation noticed  
726 with nanoindentation modulus highlights the destructuration of cell walls and not  
727 the impact of these components on the mechanical performances. In our case, other  
728 monosaccharidic components such as Glucuronic acid (0.64) or arabinose (0.15) as  
729 well as proteins (0.69) are poorly correlated with mechanical properties of hemp  
730 cell walls; these components having lower structural function, compared to  
731 rhamnose or galactose for example. Due to indentation scale, which remains high  
732 compared to the parietal architecture one, and as discussed previously, a direct  
733 correlation between mechanical properties and components fraction can't be  
734 directly established but a global tendency is noticed. The increase into porosity ratio  
735 and the decrease into crystallinity degree and non-cellulosic polysaccharides are  
736 here the main contributors to the indentation stiffness decrease; they are responsible  
737 for a pronounced degradation of both parietal architecture and composition,  
738 inducing a drop into cell wall stiffness, which is especially pronounced due to the  
739 specific loading mode during an indentation test.

## 740 **Conclusion**

741 We explored the use of controlled hydro-mechanical fibre treatment processes,  
742 particularly beating, to modify the hemp fibre surface. Ultrastructural analysis  
743 revealed that beating led to fibre bundle individualisation and fibrillation; these  
744 account for the observed reduction in bundle diameter and densification of the fibre  
745 network, and subsequent increase in macroporosity and surface area, though

746 decreased nanoporosity. We expect these to enhance physical interactions between  
747 the fibres and the matrix for composite applications.  
748 However, the beating treatment also influenced the fibres' biochemical properties.  
749 While initial hydro-treatment (soaking-drying) increased cellulose crystallinity  
750 substantially and extracted amorphous components such as pectins and  
751 hemicelluloses, subsequent beating cycles led to a drop in crystallinity probably due  
752 to defect formation.  
753 The combination of changes in structural and biochemical properties led to the  
754 observed evolution in thermal, moisture absorption and nanomechanical properties.  
755 Specifically, thermal stability and water retention capacity improved with beating  
756 treatment. Indentation modulus increased upon hydro-treatment, but subsequently  
757 almost halved to 8.4 GPa after 20 mins of beating. A Pearson's correlation analysis  
758 determined that the indentation modulus was most correlated with cellulose  
759 crystallinity and the content of three non-cellulosic cell wall polysaccharides:  
760 rhamnose, galactose and mannose. The solubilisation of these polysaccharides may  
761 have reduced the microfibril angle.

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